INDUCTION OF KIDNEY TUBULE FORMATION

Statement as to Federally Sponsored Research

This invention was funded in part by the U.S. Government under grant number HD30249 awarded by the National Institutes of Health. The Government has certain rights in the invention.

Background of the Invention

Kidney and urinary tract diseases are major causes of illness and death in the United States resulting in about 50,000 deaths per year. Renal cell carcinoma is the most common type of kidney cancer; this type of cancer affects the lining of the renal tubule and is often metastatic. About one third of the cases diagnosed show metastasis, e.g., to the lung or other organs, at the time of diagnosis. Other types of medical conditions, such as diabetes mellitus and high blood pressure, can lead to chronic kidney failure. Current therapeutic approaches include dialysis and transplantation.

Summary of the Invention

The invention provides a method of regenerating kidney tissue and is based on the discovery that Wnt-4 is sufficient to trigger kidney tubulogenesis, whereas Wnt-11 (which is also involved in tubule formation) is not. Kidney tubule formation in a post-natal mammal is stimulated by administering to the mammal a substantially pure Wnt polypeptide or a Wnt agonist. Preferably, the Wnt polypeptide is Wnt-4 or a Wnt-1 class polypeptide such as Wnt-1, Wnt-2, Wnt-3a, Wnt-7a, and Wnt-7b. A Wnt-1 class polypeptide is a Wnt polypeptide that transforms C57MG cells in culture. More preferably, the polypeptide is Wnt-3a, Wnt-4, Wnt-7a, and Wnt-7b, but not members of the Wnt-5a class of proteins such as Wnt-5a or Wnt-11. For example, the Wnt polypeptide is Wnt-4, and the Wnt

agonist is HLDAT86. Wnt-4 mediated-tubulogenesis requires cell contact; accordingly, Wnt compositions are preferably administered to kidney cells in the context of the kidney organ or in a situation in which the cells expressing a Wnt polypeptide or agonist are in close contact with cells involved in tubule formation. In preferred embodiments, sulfated glycosaminoglycans (sGAGs) are co-administered with the Wnt compositions.

The mammal to be treated is characterized as suffering from a kidney disorder. Preferably, the mammal is a human, mouse, rat, guinea pig, cow, sheep, horse, pig, rabbit, monkey, dog, or cat. The method is therapeutic or preventative and is administered to a juvenile or adult mammal. Kidney disorders include chronic renal failure, renal cell carcinoma, polycystic kidney disease, chronic obstructive uropathy, and virus-induced nephropathy. For example, the method is used to treat or prevent renal tubule epithelial cell degeneration associated with HIV-1 infection.

Administration of the Wnt compositions is local or systemic. For example, the polypeptide or Wnt agonist is administered locally to a renal tissue by, e.g., retrograde perfusion of renal tissue via blood vessels or urine collecting ducts. Wnt compositions are also administered *ex vivo* to an explanted renal tissue. For example, a kidney is removed from an individual and treated *in vitro* with a Wnt composition (e.g, a substantially pure polypeptide or an isolated nucleic acid) and then returned to the body of the same individual or a different individual.

The Wnt composition is a peptide mimetic, e.g., a polypeptide that is more resistant to proteolytic cleavage compared to a naturally-occurring Wnt polypeptide. The Wnt polypeptide is preferably soluble under physiological conditions. Accordingly, the

polypeptide is modified to improve its solubility. Alternatively, the Wnt polypeptide is present on the surface of a cell. The method utilizes a Wnt polypeptide that includes an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO:1, 2, 3, 4, or 5, a Wnt polypeptide that includes an amino acid sequence that is at least 90% identical to the amino acid sequence of SEQ ID NO:1, 2, 3, 4, or 5, a Wnt polypeptide that includes an amino acid sequence that is at least 95% identical to the amino acid sequence of SEQ ID NO:1, 2, 3, 4, or 5, and a Wnt polypeptide that includes an amino acid sequence that is identical to the amino acid sequence of SEQ ID NO:1, 2, 3, 4, or 5. The Wnt polypeptide preferably has an amino acid sequence at least 85% identical to SEQ ID NO: and functions to stimulate tubulogenesis. For example, the polypeptide may be a fragment of Wnt that stimulates tubulogenesis. A fragment has an amino acid sequence that is identical to part, but not all, of the amino acid sequence of a naturally-occurring Wnt polypeptide. A fragment of a naturally-occurring Wnt polypeptide that stimulates tubulogenesis preferably includes the amino acid sequence of at least the amino-terminal 50% of the naturally-occurring polypeptide. More preferably, the fragment contains the amino acid sequence of at least the amino terminal 75% of a naturally-occurring Wnt polypeptide. For example, the fragment contains at least residues 1-180 of naturally-occurring Wnt-1 (SEQ ID NO:1). Other fragments of Wnt polypeptides which have been shown to stimulate tubulogenesis, e.g., residues 100-331 of naturally-occurring Wnt-7a (SEQ ID NO:4, highlighted in bold), are administered. Full-length Wnt polypeptides or fragments thereof are chemically or recombinantly linked to Ig to yield Wnt-Ig fusion proteins. Human or mouse Wnt polypeptides are administered to mammals to stimulate

tubulogenesis.

Also within the invention is a method of stimulating kidney tubule formation in a post-natal mammal by administering a substantially pure or isolated nucleic acid encoding a Wnt polypeptide (e.g., a nucleic acid having the nucleotide sequence of SEQ ID NO:10, 11, or 12) or a Wnt agonist. Nucleic acids that encode a Wnt polypeptide and that have a sequence that is substantially identical to a Wnt-encoding nucleic acid sequence are administered to diseased kidney tissue.

Polypeptides or other compounds of interest are said to be "substantially pure" when they are within preparations that are at least 60% by weight (dry weight) the compound of interest. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight the compound of interest. Purity can be measured by any appropriate standard method, for example, by column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

A polypeptide or nucleic acid molecule which is "substantially identical" to a given reference polypeptide or nucleic acid molecule is a polypeptide or nucleic acid molecule having a sequence that has at least 85%, preferably 90%, and more preferably 95%, 98%, 99% or more identity to the sequence of the given reference polypeptide sequence or nucleic acid molecule. "Identity" has an art-recognized meaning and is calculated using well known published techniques, e.g., Computational Molecular Biology, 1988, Lesk A.M., ed., Oxford University Press, New York; Biocomputing: Informatics and Genome Projects, 1993, Smith, D.W., ed., Academic Press, New York; Computer Analysis of Sequence Data, Part I, 1994, Griffin, A.M. and Griffin, H.G., eds, Humana Press, New Jersey; Sequence Analysis in Molecular Biology, 1987, Heinje, G., Academic Press, New York; and

Sequence Analysis Primer, 1991, Gribskov, M. and Devereux, J., eds., Stockton Press, New York). While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans and has a definite meaning with respect to a given specified method. Sequence identity is measured using the Sequence Analysis Software Package of the Genetics Computer Group (GCS), University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705), with the default parameters as specified therein.

By "isolated nucleic acid molecule" is meant a nucleic acid molecule that is free of the genes which, in the naturally-occurring genome of the organism, flank a gene encoding a Wnt polypeptide. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or which exists as a separate molecule (e.g., a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence such as an immunoglobulin polypeptide. The term excludes large segments of genomic DNA, e.g., such as those present in cosmid clones, which contain a gene of interest flanked by one or more other genes which naturally flank it in a naturally-occurring genome.

Nucleic acid molecules include both RNA and DNA, including cDNA, genomic DNA, and synthetic (e.g., chemically synthesized) DNA. Where single-stranded, the nucleic acid molecule may be a sense strand or an antisense strand. The term therefore includes, for example, a recombinant DNA which is incorporated into a

vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote at a site other than its natural site; or which exists as a separate molecule (e.g., a cDNA or a genomic or cDNA fragment produced by polymerase chain reaction (PCR) or restriction endonuclease digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence such as an Ig polypeptide.

Wnt nucleic acids (encoding Wnt polypeptides) which hybridize at high stringency to naturally-occurring Wnt-encoding sequences are also administered to stimulate tubulogenesis. Hybridization is carried out using standard techniques such as those described in Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, (1989). "High stringency" refers to DNA hybridization and wash conditions characterized by high temperature and low salt concentration, e.g., wash conditions of 65° C at a salt concentration of approximately 0.1 X SSC. "Low" to "moderate" stringency refers to DNA hybridization and wash conditions characterized by low temperature and high salt concentration, e.g. wash conditions of less than 60° C at a salt concentration of at least 1.0 X SSC. For example, high stringency conditions may include hybridization at about 42°C, and about 50% formamide; a first wash at about 65°C, about 2X SSC, and 1% SDS; followed by a second wash at about 65°C and about 0.1% x SSC. Lower stringency conditions suitable for detecting DNA sequences having about 50% sequence identity to *csa-1* gene are detected by, for example, hybridization at about 42°C in the absence of formamide; a first wash at about 42°C, about 6X SSC, and about 1% SDS; and a second wash at about 50°C, about 6X SSC, and about 1% SDS.

The invention also includes an *ex vivo* mammalian kidney containing an exogenous Wnt polypeptide, e.g., having been bathed in or perfused with a solution containing a Wnt polypeptide or agonist. Alternatively, the *ex vivo* mammalian kidney contains exogenous DNA encoding a Wnt polypeptide. The kidney is bathed or perfused with a solution containing a Wnt-encoding nucleic acid, and cells of the kidney take up the DNA. The cells then express and secrete the recombinant Wnt polypeptide or agonist. For expression of recombinant Wnt polypeptides, Wnt-encoding sequences are operably linked to regulatory sequences, e.g., tissue specific promoters. Kidney-specific promoters are known in the art and include, e.g., the Pax-2 promoter, the cRET promoter, and the Hox b7 promoter. By "operably linked" is meant able to promote transcription of an mRNA corresponding to a polypeptide-encoding sequence located downstream on the same DNA strand.

Description of the Preferred Embodiments

A Wnt polypeptide, e.g., Wnt-4, Wnt-1, Wnt-3a, Wnt-7a and Wnt-7b, acts as a trigger to start an intrinsic program in the mesenchymal cells which then proceed to form complex nephron like structures. Wnt-4 is a secreted glycoprotein which is required for kidney tubule formation. Development of the mammalian kidney is initiated by ingrowth of the ureteric bud into the metanephric blastema. In response to signal(s) from the ureter, mesenchymal cells condense, aggregate into pretubular clusters, and undergo epithelialisation to form simple epithelial tubules. Subsequent morphogenesis and differentiation of the tubular epithelium lead to the establishment of a functional nephron.

Table 6: Human Wnt-1 amino acid sequence

1 MGLWALLPGW VSATLLLALA ALPAALAANS SGRWWGIVNV ASSTNLLTDS
KSLQLVLEPS

61 LQLLSRKQRR LIRQNPGILH SVSGGLQSAV RECKWQFRNR RWNCPTAPGP HLFGKIVNRG
121 CRETAFIFAI TSAGVTHSVA RSCSEGSIES CTCDYRRRGP GGPDWHWGGC SDNIDFGRLF
181 GREFVDSGEK GRDLRFLMNL HNNEAGRTTV FSEMRQECKC HGMSGSCTVR TCWMRLPTLR
241 AVGDVLRDRF DGASRVLYGN RGSNRASRAE LLRLEPEDPA HKPPSPHDLV YFEKSPNFCT
301 YSGRLGTAGT AGRACNSSSP ALDGCELLCC GRGHRTRTQR VTERCNCTFH WCCHVSCRNC
361 THTRVLHECL (SEQ ID NO:1)

Table 7: Human Wnt-3a amino acid sequence

CKCHGLSGSC EVKTCWWSQP DFRAIGDFLK DKYDSASEMV VEKHRESRGW
VETLRPRYTY FKVPTERDLV YYEASPNFCE PNPETGSFGT RDRTCNVSSH
GIDGCDLLCC GRGHNARAER RREKCRCVFH WCC (SEQ ID NO:2)

Table 8: Human Wnt-4 amino acid sequence

CKCH GVSGSCEVKT CWRAVPPFRQ VGHALKEKFD GATEVEPRRV GSSRALVPRN AQFKPHTDED
LVYLEPSPDF CEQDMRSGVL GTRGRTCNKT SKAIDGCELL CCGRGFHTAQ VELAERCSCK
FHWCLFLSR (SEQ ID NO:3)

Table 9: Human Wnt-7a amino acid sequence

1 MNRKALRCLG HLFLSLGMVC LRIGGFSSVV ALGATIICNK IPGLAPRQRA ICQSRPDAII
61 VIGEGSQMGL DECQFQFRNG RWNCSALGER TVFGKELKVG SRDGAFTYAI IAAGVAHAIT
121 AACTHGNLSD CGCDKEKQGQ YHRDEGWKWG GCSADIRYGI GFAKVFVDAR EIKQNARTLM
181 NLHNNEAGRK ILEENMKLEC **KCHGVSGSCT TKTCWTTLPQ FRELGYVLKD KYNEAVHVEP**
241 VRASRNKRPT FLKIKKPLSY RKPMDTDLVY IEKSPNYCEE DPVTGSVGTQ GRACNKTAPQ
301 ASGCDLMCCG RGYNTHQYAR VWQCNCKFHW CCYVKCNTCS ERTEMYTCK
(SEQ ID NO:4)

Table 10: Human Wnt-7b partial amino acid sequence

VKC GVSGSCTTKT CWTTLPKFRE VGHLLKEKYN AAVQVEVVRA SRLRQPTFLR IKQLRSYQKP
METDLVYIEK SPNYCEEDAA TGSVGTQGRI CNRTSPGADG CDTMCCGRGY NTHQYTKVWQ
CNCKFHWCCS (SEQ ID NO:5)

Table 11: Human Wnt-1 Nucleotide Sequence

1 atgtatgtat gtatgtatgt atgtatgtat acgtgcgtgc acctgtgtgt gcttggtgtc
61 agtggggctc agacatcacc tgattccctg gaactggagt tacaggtggc tataagccac
121 cacttgggtg ctgagaacag agtccgggcc tctggcagag cagtcagtgc ttttagccac
181 tgagccactc tcatcccccc aattatgttc atcttgagtt gggcaggtac ggtggcggaa
241 taggcctgta atcccagcag tcactggacc atcatgggtt ctacatatta aacctttatg
301 ttaggtaggg tcacacagca agatccggtc acaaaaccag caacaacaaa aaccaaaagg
361 agccagcttc ttcccacaag cattctttcc ctcaggtctt cagctccatc tgacagctac
421 tcggctggtg gtcctatcct ttctgagcct agttgccaga gaaacaagcc cggttcatct
481 tcatgactag cacatctaat gataagcaca ggttgactca aggtgccata gagtgacact
541 aggtacccag agcgacagaa tgacacctat gagtgcacgt cgttaatcac aaacacacac
601 acacacacac acacacacac acacacacac tcatgcaccc acctgcaaac acaattgcag
661 ccttctggac gtctcctgtc acagccccac ctccttcctg atacactgcg ttaagtggtg
721 actgtaacaa aatgacttca tgctctccct gtcctgagcc aaattacaca attatttgga
781 aagggctcaa aatgttcttc gttagaagtt tctggataca ccaatacaca ggagcgtgca
841 ccctcagaac acatgtacac tttgacttaa tctcacgggt gacacaccga cgcttacact
901 ccccctagcc cacagaggca aactgctggg cgcttctgag tttctcactg ccaccagctc
961 ggtttgctca gcctaccccc gcacccccgcg cccgggaatc cctgaccaca gctccaccca
1021 tgctctgtct ccttcttttc cttctctgtc cagccgtcgg ggttcctggg tgaggaagtg
1081 tctccacgga gtcgctggct agaaccacaa ctttcatcct gccattcaga atagggaaga
1141 gaagagacca cagcgtaggg gggacagagg agacggactt cgagaggaca gccccaccgg
1201 cgcgtgtggg ggaggcaatc caggctgcaa acaggttgtc cccagcgcat tgtccccgcg

1261 ccccctggcg gatgctggtc cccgacgggc tccggacgcg cagaagagtg aggccggcgc
1321 gcgtgggagg ccatcccaag gggaggggtc ggcggccagt gcagacctgg aggcggggcc
1381 accaggcagg gggcgggggt gagccccgac ggttagcctg tcagctcttt gctcagaccg
1441 gcaagagcca cagcttcgct cgccactcat tgtctgtggc cctgaccagt gcgccctggt
1501 gcttttagtg ccgcccgggc ccggaggggc agcctcttct cactgcagtc agcgccgcaa
1561 ctataagagg cctataagag gcggtgcctc ccgcagtggc tgcttcagcc cagcagccag
1621 gacagcgaac catgctgcct gcggcccgcc tccagactta ttagagccag cctgggaact
1681 cgcatcactg ccctcaccgc tgtgtccagt cccaccgtcg cggacagcaa ccacagtcgt
1741 cagaaccgca gcacagaacc agcaaggcca ggcaggccat ggggctctgg gcgctgctgc
1801 ccagctgggt ttctactacg ttgctactgg cactgaccgc tctgcccgca gccctggctg
1861 ccaacagtag tggccgatgg tggtaagtga gctagtacgg ggtccgccac ttgtcctggg
1921 gcaaagagcc aggcacgggc cttacccagc tcccacgctg tggggatcac caacctacag
1981 acccccctcg tgcattgtga cttcacatcc agggtgctca cacctagaac tagctctgct
2041 gaagtggggc acatcattgg catgcagaag cccagataca ccaggctcag agaccattcc
2101 catttaatac gaccccgttt ctgctgagca acaggtccca acctcgctgt ggtgggtgct
2161 caggtgtccc ttaggtcttg aaccaaaaaa aaaaaaaaaa aaaaaaaaaa accagatatt
2221 agctttgagg tgagggagtg gaattcctaa gtttttcaag gtgggcaagg ctgcaggtgg
2281 ggtttctcct cgggggctga cttgaagaaa ggaagagcta aggtagccat gccttttctg
2341 tccactcact agactctgga gctcagggcc aggcaaggat agggtggtac agcctgtatg
2401 gttaggatgc aggtcccctc ccctggactg aacccttatg catcccgcca ggggcatcgt
2461 gaacatagcc tcctccacga acctgttgac ggattccaag agtctgcagc tggtgctcga
2521 gcccagtctg cagctgctga gccgcaagca gcggcgactg atccgacaga acccggggat

2581 cctgcacagc gtgagtggag ggctccagag cgctgtgcga gagtgcaaat
ggcaattccg
2641 aaaccgccgc tggaactgcc ccactgctcc ggggccccac ctcttcggca
agatcgtcaa
2701 ccgaggtggg tgcccaggaa agcgacgctt ccgggattaa gggaaaagca
gggtcatctc
2761 cagggcatag gcgggcgaag gcagggaaga catcccaggg ttatatgtga
tcaaactgag
2821 aatcgcctgg tgccggcagt taccgtaggt cagcaccaga ttctttctag
ccttgcgttg
2881 tgagcatgat ctttaacgtt gctggccact ggcccacaga aagggaattc
cggatcgtgg
2941 gcgctgggcg acagctgttt ttccctagcc ttcctcaaag gtacctggga
agctgatctc
3001 tgagggctag ctagggttgt gcttcgcacc cagcaaagtt tgcactgcca
atactagtag
3061 cgatcttggc tatgcagatt tgttctactt gggaatctcc ccttggagct
gctctgctag
3121 ggctctggag tctcagtaaa gcttagagag gagggcattc catgcttcgc
acacatgact
3181 ccaaggatgt tggactgtag ggtaccaagt cttccaaaca gggtgctgag
ttggccccac
3241 gccttctctc aactgatgcg ggtcgcttc acccacaggc tgccgagaaa
cagcgttcat
3301 cttcgcaatc acctccgccg gggtcacaca ttccgtggcg cgctcctgct
ccgaaggctc
3361 catcgagtcc tgcacctgcg actaccggcg gcgcggccct gggggccccg
actggcactg
3421 ggggggctgc agtgacaaca tcgattttgg tcgcctcttt ggccgagagt
tcgtggactc
3481 cggggagaag gggcgggacc tacgcttcct catgaacctt cacaacaacg
aggcagggcg
3541 aacggtacgt cggtgtgtcc ggaaccaatg gcaggggaga tgtaagacag
gtgcacgggg
3601 acagaggcac agggaggggc ttcccgagag agtgggactc taggagggaa
gacagagaag
3661 aggtggtggt tgagggcaaa gaggttcctg agctgatgac agaacagaag
agattagcag
3721 gctatcaaca cgtgggatgt attgagatgg ctccatggca cacttttgaa
agataaaagt
3781 gacttgctgg cgtggagcag agtctggccg aatgtcccta tctcagcggg
ccattttgca
3841 cttcctctct cccgagctta gtcacacctg gaccttggct gaagtttcca
cagcatcgac

3901 gtgacccggg tggggtgggg gtggggaagt atgggtggtg gttcgtggga tgttggcttt
3961 gaccttttct tccctcctcc cctcgtcccc tcctccccca gaccgtgttc tctgagatgc
4021 gccaagagtg caaatgccac gggatgtccg gctcctgcac ggtgcgcacg tgttggatgc
4081 ggctgcccac gctgcgcgct gtgggcgacg tgctgcgcga ccgcttcgac ggcgcctccc
4141 gcgtccttta cggcaaccga ggcagcaacc gcgcctcgcg ggcggagctg ctgcgcctgg
4201 agcccgaaga ccccgcgcac aagcctccct cccctcacga cctcgtctac ttcgagaaat
4261 cgcccaactt ctgcacgtac agtggccgcc tgggcacagc tggcacagct ggacgagctt
4321 gcaacagctc gtctcccgcg ctggacggct gtgagctgct gtgctgtggc cgaggccacc
4381 gcacgcgcac gcagcgcgtc acggagcgct gcaactgcac cttccactgg tgctgccacg
4441 tcagctgccg caactgcacg cacacgcgcg ttctgcacga gtgtctatga ggtgccgcgc
4501 ctccgggaac gggaacgctc tcttccagtt ctcagacaca ctcgctggtc ctgatgtttg
4561 cccaccctac cgcgtccagc cacagtccca gggttcatag cgatccatct ctcccacctc
4621 ctacctgggg actcctgaaa ccacttgcct gagtcggctc gaaccctttt gccatcctga
4681 gggccctgac ccagcctacc tccctccctc tttgagggag actccttttg cactgccccc
4741 caatttggcc agagggtgag agaaagattc ttcttctggg gtgggggtgg ggaggtcaac
4801 tcttgaaggt gttgcggttc ctgatgtatt ttgcgctgtg acctctttgg gtattatcac
4861 ctttccttgt ctctcgggtc cctataggtc ccttgagttc tctaaccagc acctctgggc
4921 ttcaaggcct ttcccctccc acctgtagct gaagagtttc cgagttgaaa gggcacggaa
4981 agctaagtgg gaaaggaggt tgctggaccc agcagcaaaa ccctacattc tccttgtctc
5041 tgcctcggag ccattgaaca gctgtgaacc atgcctccct cagcctcctc ccaccccttc
5101 ctgtcctgcc tcctcatcac tgtgtaaata atttgcaccg aaatgtggcc gcagagccac
5161 gcgttcggtt atgtaaataa aactatttat tgtgctgggt tccagcctgg gttgcagaga

5221 ccaccctcac cccacctcac tgctcctctg ttctgctcgc cagtcctttt gttatccgac
5281 ctttttctc ttttacccag cttctcatag gcgcccttgc ccaccggatc agtatttcct
5341 tccactgtag ctattagtgg ctcctcgccc ccaccaatgt agtatcttcc tctgaggaat
5401 aaaatatcta tttttatcaa cgactctggt ccttgaatcc agaacacagc atggcttcca
5461 acgtcctctt cccttccaat ggacttgctt ctcttctcat agccaaacaa aagagataga
5521 gttgttgaag atctcttttc cagggcctga gcaaggaccc tgagatcctg accccttggat
5581 gaccctaaat gagaccaact agggatc (SEQ ID NO:6)

Table 12: Human Wnt-2 Nucleotide Sequence

1 agcagagcgg acgggcgcgc gggaggcgcg cagagctttc gggctgcagg cgctcgctgc
61 cgctggggaa ttgggctgtg ggcgaggcgg tccgggctgg cctttatcgc tcgctgggcc
121 catcgtttga aactttatca gcgagtcgcc actcgtcgca ggaccgagcg gggggcgggg
181 gcgcggcgag gcggcggccg tgacgaggcg ctcccggagc tgagcgcttc tgctctgggc
241 acgcatggcg cccgcacacg gagtctgacc tgatgcagac gcaaggggt taatatgaac
301 gcccctctcg gtggaatctg gctctggctc cctctgctct tgacctggct cacccccgag
361 gtcaactctt catggtggta catgagagct acaggtggct cctccagggt gatgtgcgat
421 aatgtgccag gcctggtgag cagccagcgg cagctgtgtc accgacatcc agatgtgatg
481 cgtgccatta gccagggcgt ggccgagtgg acagcagaat gccagcacca gttccgccag
541 caccgctgga attgcaacac cctggacagg gatcacagcc tttttggcag ggtcctactc
601 cgaagtagtc gggaatctgc ctttgtttat gccatctcct cagctggagt tgtatttgcc
661 atcaccaggg cctgtagcca aggagaagta aaatcctgtt cctgtgatcc aaagaagatg
721 ggaagcgcca aggacagcaa aggcattttt gattggggtg gctgcagtga taacattgac
781 tatgggatca aatttgcccg cgcatttgtg gatgcaaagg aaaggaaagg aaaggatgcc
841 agagccctga tgaatcttca caacaacaga gctggcagga aggctgtaaa gcggttcttg
901 aaacaagagt gcaagtgcca cggggtgagc ggctcatgta ctctcaggac atgctggctg
961 gccatggccg acttcaggaa aacgggcgat tatctctgga ggaagtacaa tggggccatc
1021 caggtggtca tgaaccagga tggcacaggt ttcactgtgg ctaacgagag gtttaagaag
1081 ccaacgaaaa atgacctcgt gtattttgag aattctccag actactgtat cagggaccga
1141 gaggcaggct ccctgggtac agcaggccgt gtgtgcaacc tgacttcccg gggcatggac
1201 agctgtgaag tcatgtgctg tgggagaggc tacgacacct cccatgtcac ccggatgacc
1261 aagtgtgggt gtaagttcca ctggtgctgc gccgtgcgct gtcaggactg cctggaagct
1321 ctggatgtgc acacatgcaa ggcccccaag aacgctgact ggacaaccgc tacatgaccc
1381 cagcaggcgt caccatccac cttcccttct acaaggactc cattggatct gcaagaacac
1441 tggacctttg ggttctttct gggggatat ttcctaaggc atgtggcctt tatctcaacg
1501 gaagcccct cttcctccct gggggcccca ggatgggggg ccacacgctg cacctaaagc
1561 ctaccctatt ctatccatct cctggtgttc tgcagtcatc tcccctcctg gcgagttctc

1621 tttggaaata gcatgacagg ctgttcagcc gggagggtgg tgggcccaga ccactgtctc
1681 cacccacctt gacgtttctt ctttctagag cagttggcca agcagaaaaa aaagtgtctc
1741 aaaggagctt tctcaatgtc ttcccacaaa tggtcccaat taagaaattc catacttctc
1801 tcagatggaa cagtaaagaa agcagaatca actgcccctg acttaacttt aacttttgaa
1861 aagaccaaga cttttgtctg tacaagtggt tttacagcta ccacccttag ggtaattggt
1921 aattacctgg agaagaatgg ctttcaatac ccttttaagt ttaaaatgtg tatttttcaa
1981 ggcatttatt gccatattaa aatctgatgt aacaaggtgg ggacgtgtgt cctttggtac
2041 tatggtgtgt tgtatctttg taagagcaaa agcctcagaa agggattgct ttgcattact
2101 gtccccttga tataaaaaat ctttagggaa tgagagttcc ttctcactta gaatctgaag
2161 ggaattaaaa agaagatgaa tggtctggca atattctgta actattgggt gaatatggtg
2221 gaaaataatt tagtggatgg aatatcagaa gtatatctgt acagatcaag aaaaaaagga
2281 agaataaaat tcctatatca t (SEQ ID NO:7)

Table 13: Murine Wnt-3A Nucleotide Sequence

1 gaattcatgt cttacggtca aggcagaggg cccagcgcca ctgcagccgc gccacctccc
61 agggccgggc cagcccaggc gtccgcgctc tcggggtgga ctcccccgc tgcgcgctca
121 agccggcgat ggctcctctc ggatacctct tagtgctctg cagcctgaag caggctctgg
181 gcagctaccc gatctggtgg tccttggctg tgggacccca gtactcctct ctgagcactc
241 agcccattct ctgtgccagc atcccaggcc tggtaccgaa gcagctgcgc ttctgcagga
301 actacgtgga gatcatgccc agcgtggctg agggtgtcaa agcgggcatc caggagtgcc
361 agcaccagtt ccgaggccgg cgttggaact gcaccaccgt cagcaacagc ctggccatct
421 ttggccctgt tctggacaaa gccacccggg agtcagcctt tgtccatgcc atcgcctccg
481 ctggagtagc tttcgcagtg acacgctcct gtgcagaggg atcagctgct atctgtgggt
541 gcagcagccg cctccagggc tccccaggcg agggctggaa gtggggcggc tgtagtgagg
601 acattgaatt tggaggaatg gtctctcggg agtttgccga tgccagggag aaccggccgg
661 atgcccgctc tgccatgaac cgtcacaaca atgaggctgg gcgccaggcc atcgccagtc
721 acatgcacct caagtgcaaa tgccacgggc tatctggcag ctgtgaagtg aagacctgct
781 ggtggtcgca gccggacttc cgcaccatcg ggatttcct caaggacaag tatgacagtg
841 cctcggagat ggtggtagag aaacaccgag agtctcgtgg ctgggtggag

accctgaggc
901 cacgttacac gtacttcaag gtgccgacag aacgcgacct ggtctactac gaggcctcac
961 ccaacttctg cgaacctaac cccgaaaccg gctccttcgg gacgcgtgac cgcacctgca
1021 atgtgagctc gcatggcata gatgggtgcg acctgttgtg ctgcgggcgc gggcataacg
1081 cgcgcactga gcgacggagg gagaaatgcc actgtgtttt ccattggtgc tgctacgtca
1141 gctgccagga gtgcacacgt gtctatgacg tgcacacctg caagtaggag agctcctaac
1201 acgggagcag ggttcattcc gaggggcaag gttcctacct gggggcgggg ttcctacttg
1261 gaggggtctc ttacttgggg actcggttct tacttgaggg cggagatcct acctgtgagg
1321 gtctcatacc taaggacccg gtttctgcct tcagcctggg ctcctatttg ggatctgggt
1381 tcctttttag gggagaagct cctgtctggg atacgggttt ctgcccgagg gtggggctcc
1441 acttggggat ggaattccaa tttgggccgg aagtcctacc tcaatggctt ggactcctct
1501 cttgacccga cagggctcaa atggagacag gtaagctact ccctcaacta ggtggggttc
1561 gtgcggatgg gtgggagggg agagattagg gtccctcctc ccagaggcac tgctctatct
1621 agatacatga gagggtgctt cagggtgggc cctatttggg cttgaggatc ccgtgggggc
1681 ggggcttcac cccgactggg tggaactttt ggagaccccc ttccactggg gcaaggcttc
1741 actgaagact catgggatgg agctccacgg aaggaggagt tcctgagcga gcctgggctc
1801 tgagcaggcc atccagctcc catctggccc ctttccagtc ctggtgtaag gttcaacctg
1861 caagcctcat ctgcgcagag caggatctcc tggcagaatg aggcatggag aagaactcag
1921 gggtgatacc aagacctaac aaaccccgtg cctgggtacc tcttttaaag ctctgcaccc
1981 cttcttcaag ggctttccta gtctccttgg cagagctttc ctgaggaaga tttgcagtcc
2041 cccagagttc aagtgaacac ccatagaaca gaacagactc tatcctgagt agagagggtt
2101 ctctaggaat ctctatgggg actgctagga aggatcctgg gcatgacagc ctcgtatgat
2161 agcctgcatc cgctctgaca cttaatactc agatctcccg ggaaacccag

ctcatccggt
2221 ccgtgatgtc catgccccaa atgcctcaga gatgttgcct cactttgagt
tgtatgaact
2281 tcggagacat ggggacacag tcaagccgca gagccagggt tgtttcagga
cccatctgat
2341 tccccagagc ctgctgttga ggcaatggtc accagatccg ttggccacca
ccctgtcccg
2401 agcttctcta gtgtctgtct ggcctggaag tgaggtgcta catacagccc
atctgccaca
2461 agagcttcct gattggtacc actgtgaacc gtccctcccc ctccagacag
gggaggggat
2521 gtggccatac aggagtgtgc ccggagagcg cggaaagagg aagagaggct
gcacacgcgt
2581 ggtgactgac tgtcttctgc ctggaacttt gcgttcgcgc ttgtaacttt
attttcaatg
2641 ctgctatatc cacccaccac tggatttaga caaaagtgat tttctttttt
tttttttctt
2701 ttctttctat gaaagaaatt attttagttt atagtatgtt tgtttcaaat
aatggggaaa
2761 gtaaaaagag agaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaa
(SEQ ID NO:8)

Table 14: Human Wnt-3a nucleotide sequence

tgtaagtgcc acgggctgtc gggcagctgc gaggtgaaga catgctggtg
gtcgcaaccc gacttccgcg ccatcggtga cttcctcaag gacaagtacg
acagcgcctc ggagatggtg gtggagaagc accgggagtc ccgcggctgg
gtggagaccc tgcggccgcg ctacacctac ttcaaggtgc ccacggagcg
cgacctggtc tactacgagg cctcgcccaa cttctgcgag cccaaccctg
agacgggctc cttcggcacg cgcgaccgca cctgcaacgt cagctcgcac
ggcatcgacg gctgcgacct gctgtgctgc ggccgcggcc acaacgcgcg
agcggagcgg cgccgggaga agtgccgctg cgtgtttcac tggtgctgt
(SEQ ID NO:9)

Human nucleic acid sequences which encode Wnt-4, Wnt-7a, and Wnt-7b are shown Tables 15, 16, and 17, respectively.

Human and mouse Wnt polypeptides function similarly in transformation assays. Accordingly, human or mouse Wnt polypeptides or nucleic acids are administered to mammals to therapeutically stimulate

tubulogenesis. The amino acid and nucleotide sequences of Wnt polypeptides are known in the art, e.g., human Wnt-1 (GENBANK® X03072), human Wnt-2 (GENBANK® X07876), human Wnt-4 (GENBANK® AAB30677), human Wnt-7a (GENBANK® 000755), mouse Wnt-1 (GENBANK® P04426), mouse Wnt-2 (GENBANK® P21552), mouse Wnt-3a (GENBANK® P27467), mouse Wnt-4 (GENBANK® P22724 and M89787), mouse Wnt-7a (GENBANK® M89802), and mouse Wnt-7a (GENBANK® M89801).

Kidney tubulogenesis is a multi-step process with a hierarchy of signaling systems. A permissive signal from the ureter to the mesenchyme triggers survival and tubulogenesis in the mesenchyme, signals from the mesenchyme to the ureter are required for proliferation and branching morphogenesis of the ureter. Other signaling systems within the ureter are required for local adhesion and proliferation, changes which may mediate branching morphogenesis, and within the mesenchyme, for tubulogenesis as evidenced by the role of Wnt-4.

The data described herein indicate that Wnt-4 is sufficient to trigger tubulogenesis in isolated metanephric mesenchyme, whereas Wnt-11 which is expressed in the tip of the growing ureter is not. Wnt-4 signaling depends on cell contact and sulphated glycosaminoglycans. Wnt-4 is required for triggering tubulogenesis but not for later developmental events. The Wnt-4 signal can be replaced by other members of the Wnt gene family including Wnt-1, Wnt-3a, Wnt-7a and Wnt-7b. Further, dorsal spinal cord, which has been thought to mimic ureteric signaling in tubule induction, induces Wnt-4 mutant as well as wild-type mesenchyme suggesting that spinal cord derived signal(s) likely act by mimicking the normal mesenchymal action of Wnt-4. These results indicate that Wnt-4 is a key auto-regulator of the mesenchymal to epithelial transformation that leads to

tubulogenesis and nephrogenesis.

<u>Therapeutic administration of a Wnt polypeptide or agonist</u>

Wnt polypeptides or agonists are useful to treat kidney disorders such as chronic renal insufficiency, end-stage chronic renal failure, glomerulonephritis, glomerulosclerosis, interstitial nephritis, pyelonephritis, kidney failure due to viral disease, kidney failure after transplantation.

Wnt polypeptides are at least about 10 amino acids, usually about 20 contiguous amino acids, preferably at least 40 contiguous amino acids, more preferably at least 50 contiguous amino acids, and most preferably at least about 60 to 80 contiguous amino acids in length and have the biological activity of triggering tubulogenesis. For example, a Wnt polypeptide is at least 50% of the length of the corresponding naturally-occurring Wnt polypeptide and has the amino acid sequences of the amino-terminal half of the naturally-occurring polypeptide. Such peptides are generated by methods known to those skilled in the art, including proteolytic cleavage of the protein, *de novo* synthesis of the fragment, or genetic engineering, e.g., cloning and expression of a fragment of Wnt-encoding cDNA. Therapeutic compositions are administered in a pharmaceutically acceptable carrier (e.g., physiological saline). Carriers are selected on the basis of mode and route of administration and standard pharmaceutical practice. A therapeutically effective amount of a composition (e.g., Wnt polypeptide or agonist) is an amount which is capable of producing a medically desirable result, e.g., tubulogenesis, in a treated animal. As is well known in the medical arts, dosage for any one animal depends on many factors, including the animal's size, body surface area, age, the particular

compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently (e.g., other Wnt polypeptides) is 0.1 to 100 mg/kg body weight. Administration is generally be parenterally, e.g., intravenously, subcutaneously, intramuscularly, or intraperitoneally. The compositions of the invention can be administered locally i.e., at the site of organ damage or systemically. For example, the route of delivery is by intravenous infusion, localized injection or implants. The polypeptides or agonists may be formulated so as to have a continual presence in the tissue during the course of treatment, e.g., by being covalently attached to a polymer such as polyethylene glycol (PEG). Such continuous release formulations are administered at weekly intervals or at multiples of weekly intervals. Examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing the polypeptide or agonist, which matrices are in the form of shaped films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (e.g., poly(2-hydroxyethyl-methacrylate) as described by Langer et al., 1981, J. Biomed. Mater. Res., 15: 167-277 and Langer, 1982, Chem. Tech., 12: 98-105 or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman et al., 1983, Biopolymers, 22: 547-556), non-degradable ethylene-vinyl acetate (Langer et al., *supra*), degradable lactic acid-glycolic acid copolymers, polylactate polyglycolate (PLGA), and poly-D-(-)-3-hydroxybutyric acid (EP 133,988). While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid provide release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods.

Sustained-release Wnt compositions also include liposomally entrapped Wnt polypeptides or agonists. Liposomes containing Wnt compositions are prepared by methods known in the art, e.g., Epstein et al., 1985, Proc. Natl. Acad. Sci. USA, 82: 3688-3692; Hwang et al., 1980, Proc. Natl. Acad. Sci. USA, 77: 4030-4034; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. The compositions may also be administered directly to a tissue site, e.g., by biolistic delivery to an internal or external target site or by catheter into a body lumen. Therapeutic compositions are administered by retrograde perfusion of kidney via the ureter or other urine collecting lumens, e.g., using a catheter or perfusion apparatus, such as that described in U.S. Pat. No. 5,871,464.

Analogs, homologs, or mimetics of the above peptides may also be used to induce and promote kidney tubule formation in a post-natal mammal. Analogs can differ from the naturally-occurring Wnt polypeptides by amino acid sequence, or by modifications which do not affect the sequence, or both. Modifications (which do not normally alter primary sequence) include *in vivo* or *in vitro* chemical derivitization of polypeptides, e.g., acetylation or carboxylation. Also included are modifications of glycosylation, e.g., those made by modifying the glycosylation patterns of a polypeptide during its synthesis and processing or in further processing steps, e.g., by exposing the polypeptide to enzymes which affect glycosylation, e.g., mammalian glycosylating or deglycosylating enzymes. To improve the solubility and therapeutic half-life of Wnt polypeptides, Wnt-Ig fusion proteins are produced. Methods of making Ig fusion proteins is well known in the art (e.g., as described in Current Protocols of Immunology, 1994, Coligan et al., eds., John Wiley & Sons, Inc., p.

10.19.1-10.19.11).

To render the therapeutic peptides less susceptible to cleavage by peptidases, the peptide bonds of a peptide may be replaced with an alternative type of covalent bond (a "peptide mimetic"). Where proteolytic degradation of the peptides following injection into the subject is a problem, replacement of a particularly sensitive peptide bond with a noncleavable peptide mimetic renders the resulting peptide more stable, and thus more useful as a therapeutic. Such mimetics, and methods of incorporating them into polypeptides, are well known in the art. Similarly, the replacement of an L-amino acid residue with a D-amino acid is a standard way of rendering the polypeptide less sensitive to proteolysis. Also useful are amino-terminal blocking groups such as t-butyloxycarbonyl, acetyl, theyl, succinyl, methoxysuccinyl, suberyl, adipyl, azelayl, dansyl, benzyloxycarbonyl, fluorenylmethoxycarbonyl, methoxyazelayl, methoxyadipyl, methoxysuberyl, and 2,4,-dinitrophenyl. Peptides may be administered to a subject intravenously in a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers are biologically compatible vehicles which are suitable for administration to an animal: e.g., physiological saline.

Wnt polypeptides are generally administered *in vivo* to allow regeneration of kidney tissue in the context of the autologous organ. However, kidney tissue or dissociated cells (derived from kidney tissue or embryonic tissue) may be treated outside the body (i.e., *ex vivo*) and then transplanted back into the body from which it was derived or into a different mammal. In the case of *ex vivo* therapy, a damaged or diseased kidney is removed from an individual, treated with a Wnt polypeptide (or DNA encoding a Wnt polypeptide) and then transplanted into the same individual or a different

individual.

Therapeutic administration of DNA encoding a Wnt polypeptide or agonist

Gene therapy for regeneration of kidney tissue is carried out by directly administering the claimed DNA to a mammal or by transfecting kidney cells, e.g., renal mesenchymal cells or endothelial cells, with Wnt-encoding DNA *in vivo* or *ex vivo*. Gene transfer into kidney tissue is carried out using known methods, e.g., bathing the tissue or cells in a solution containing Wnt-encoding DNA. Alternatively, kidney tissue is perfused *in vivo* or explanted kidney tissue is perfused *ex vivo*, using a perfusion apparatus, such as that described in U.S. Pat. No. 5,871,464. After the cells are contacted with DNA, the cells or organ is transplanted into a recipient (or returned to the host from which it was removed). If the cells in suspension, the cells are infused into the mammal to be treated.

To express a Wnt polypeptide in a kidney cell, a Wnt-encoding DNA is introduced into a target cell, e.g., a mesenchymal or epithelial kidney cell, of the mammal by standard vectors and/or gene delivery systems. For example, expression of exogenous Wnt DNA in an epithelial cell induces production and secretion of a Wnt polypeptide, which in turn, leads to tubulogenesis and kidney regeneration. Suitable gene delivery systems may include liposomes, receptor-mediated delivery systems, naked DNA, and viral vectors such as herpes viruses, retroviruses, adenovirus, and adeno-associated virus, among others. A therapeutically effective amount is an amount of the nucleic acid of the invention which is capable of producing a medically desirable result in a treated animal, e.g., tubulogenesis.

DNA or transfected cells may be administered in a pharmaceutically acceptable carrier. Pharmaceutically

acceptable carriers are biologically compatible vehicles which are suitable for administration to a mammal, e.g., physiological saline. As is well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Dosages will vary, but a preferred dosage for intravenous administration of DNA is from approximately 10^6 to 10^{22} copies of the DNA molecule. The compositions of the invention may be administered locally or systemically. As with other therapeutic compositions such as peptides, administration of a nucleic acid composition is generally be parenterally, e.g., intravenously. DNA is also administered by retrograde perfusion of kidney tissue using, e.g., a catheter. DNA may also be administered directly to the target site, e.g., by biolistic delivery to a kidney tissue or by an implantable device.

Methods of delivering nucleic acids to kidney tissue are known in the art, e.g, those described by Sukhatme et al. in U.S. Pat. No. 5,869,230. Nucleic acids are expressed under the control of tissue-specific, e.g., kidney-specific, promoters such as the Pax-2 promoter, the cRET promoter, and the Hox b7 promoter. Promoter constructs for inducible and constitutive expression of heterologous sequences are well known in the art and commercially-available. For example, nucleic acids are expressed under the control of the cytomegalovirus (CMV) β-actin promoter for general constitutive expression.

<u>Method of screening for compounds which increase Wnt expression</u>

A screening assay to identify compounds which are capable of inducing or increasing Wnt polypeptide

expression in kidney tissue of a post-natal mammal (i.e., non-embryonic cells) is carried out as follows. For example, a sample of kidney cells, e.g., cultured mesenchymal or epithelial cells, is incubated in the presence of a candidate compound. A sample of control cells is incubated in the absence of the compound. Each sample of cells is evaluated for the expression of a Wnt polypeptide, e.g, Wnt-4. To test for presence of the Wnt gene product, each sample of cells can be incubated with a Wnt-specific antibody and the cells evaluated for binding of the antibody by methods well known in the art, e.g., immunofluorescent staining. The amount of antibody binding correlates with the level of expression of the Wnt polypeptide. Wnt expression is also measured at the level of gene transcription. For example, Wnt transcripts can be measured by Northern blotting techniques using Wnt-specific DNA probes or by PCR using Wnt-specific DNA primers. A increase in the amount of Wnt gene expression in cells contacted with a candidate compound compared to the amount in untreated cells indicates that the candidate compound is capable of inducing or increasing the expression of a Wnt polypeptide in kidney cells (and inducing tubulogenesis). The compound is tested in tissue or organ culture systems as described below to determine whether the compound triggers tubulogenesis.

<u>Mouse model of renal development</u>

Mouse renal development is characterized by the continuous interaction of epithelial and mesenchymal compartments both of which are derived from the intermediate mesenchyme. These compartments are the nephric duct and its derivative, the ureter, and the nephrogenic mesenchyme which lies adjacent to these ducts. As a consequence of these interactions, three embryonic kidneys are laid down from anterior to

posterior in time and space. While the initial organ, the pronephros is only a very transient structure established at 8-8.5 days post coitum (d.p.c.), the mesonephros extends by posterior elongation of the nephric duct and subsequent tubule induction in the adjacent mesonephrogenic mesenchyme between 9 and 11 d p.c. Although forming elaborate tubules, the mesonephros of the male never becomes a functional organ but contributes to the ductal network of the rete testis. Metanephric development is initiated when a bud emerges from the nephric duct at the level of the hind limbs around 10.5 d.p.c. The ureteric duct subsequently invades the metanephric blastema which lies at the posterior end of the intermediate mesoderm.

In a process repeated many times, mesenchymal cells condense around the tip of the ureter, i.e., bud, aggregate, epithelialize and undergo morphogenetic movements . Cellular differentiation occurs to form a major part of the nephron, the functional unit of the vertebrate kidney. The ureter continues to grow and to branch forming the collecting duct system of the mature organ. 7-10 days post partum, nephron formation ceases as the mesenchymal stem cells in the periphery of the kidney are exhausted.

The role of Wnt-11, Wnt-4 and other Wnt family members in tubule induction was studied. Wnt-4, but not Wnt-11 was found to be able to induce tubule formation, suggesting that spinal cord mediated tubulogenesis reflects the normal mesenchymal function of Wnt-4 rather than that of a ureteric bud derived signal.

The following reagents and procedures were used to evaluate Wnt signalling in the developing kidney.

Mice

Wnt-4 heterozygotes were derived and genotyped using known methods, e.g., that described by Stark et

al., 1994, Nature 372:679-683. Embryos for kidney dissections were derived from matings of Swiss Webster (SW) wild-type animals or Wnt-4 heterozygotes. For timed pregnancies, plugs were checked in the morning after mating, noon was taken as 0.5 d.p.c.

Cell lines

Cell lines which stably express various Wnt genes or LacZ were prepared using standard methods, e.g, that described by Pear et al., 1993, Proc. Natl. Acad. Sci. USA 90: 8392-8396. For Wnt polypeptide expression, full-length cDNAs encoding Wnt-1 (van Ooyen and Nusse, 1984, Cell 39: 233-240), Wnt-3a (Roelink and Nusse, 1991, Genes Dev. 5: 381-388), Wnt-4, Wnt-5a, Wnt-7a, Wnt-7b (Gavin et al., 1990, Genes Dev. 4: 2319-2332), Wnt-11 (Kispert et al., 1996, Development 122:3627-3637) and lacZ were cloned into an expression vector, e.g, the retroviral expression vector pLNCX which confers expression of foreign genes under the control of the CMV promotor. Bosc23 packaging cells were transfected with recombinant DNA constructs. Viral supernatants were collected 48-72 h later and used to infect standard NIH3T3 cells. After 10 d of selection in G418, pools of cells were used for recombination experiments. 50,000 cells were plated in 50 μl of medium on polycarbonate filter and grown for 18-24 h at 37°C in 5% CO_2.

Organ culture techniques

Metanephric kidneys from SW or Wnt-4 intercrosses were dissected in phosphate buffered saline (PBS). To generate a preparation of dissociated kidney cells from embryonic or mature tissue, the tissue is dissected and enzymatically digested. For example, metanephric mesenchyme was dissected manually from the ureter (bud stage [10.75 d.p.c.] to early T stage [11.5 d p.c.]), following a 2 min. incubation in 3% pancreatin/trypsin (GibcoBRL) in Tyrode's solution. In recombination

experiments with wild-type mesenchymes, samples were pooled before being distributed to individual experiments. In experiments with Wnt-4 mutant embryos, metanephric mesenchyme from each kidney of the embryo was kept separate. The remainder of an embryo was used for genotyping by Southern analysis. In recombination experiments with dorsal spinal cord, metanephric mesenchyme from two kidneys was surrounded by two dissected pieces of dorsal spinal cord from the same embryo on a 1 μm polycarbonate filter (Costar). For direct recombination experiments with Wnt-expressing cells, two mesenchymes were placed on top of modified NIH3T3 cells. For transfilter experiments, 50,000 cells in 50 μl medium were seeded on a 1 μm filter 18-24 h prior to the recombination. Cells were then covered with a 1 μm filter and two mesenchymes placed on this filter. Filters (4-6 mm in size) were supported by stainless steel grids on the surface of the culture medium (Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 2 mM glutamine, 1 × penicillin/streptomycin). Medium was changed every 2 d. For studies of glycosaminoglycan dependence of tubule induction, the medium was supplemented with 30 mM $NaClO_3$ after 0 h, 24 h and 48 h, respectively. In experiments concerning pore size dependence of induction, the pore size of the upper filter in the transfilter set-up was varied from 0.05 μm, 0.1 μm, 0.4 μm, 0.8 μm to 1 μm. For marker experiments, at least 6 specimens were processed.

For *in situ* hybridization analysis, filters were submerged in cold methanol for 10 seconds and then fixed in 4% paraformaldehyde in PBS overnight prior to stepwise transfer into methanol and storage at -20°C. For histological analysis, filters were fixed in Bouin's solution and stored in 70% ethanol at 4°C.

In situ hybridization analysis

In situ hybridization analysis on whole mount cultures were performed using standard methods. Full-length cDNAs for WT-1 (Pritchard-Jones et al., 1990, Nature 346:194-197), Pax-2 (Dressler et al., 1990, Development 109:787-795), Pax-8 (Plachov et al., 1990, Development 110:643-651), Wnt-4 (Gavin et al., 1990, Genes Dev. 4:2319-2332) and E-cadherin (Ringwald et al., 1987, EMBO J. 6:3647-3653) were labeled with Digoxigenin for whole mount detection.

Histological analysis and documentation

Samples were dehydrated, embedded in wax and sectioned at 5 μm. Sections were dewaxed, rehydrated and stained with haematoxylin and eosin. Brightfield images of cultures and marker stainings were taken with a binocular on Kodak 64T slide film. Histological sections were photographed on the same film on a Leitz Axiophot. Slides were scanned and documented in Adobe Photoshop 4.0.

Spinal cord mimics a mesenchymal signal for tubule induction

The identification of Wnt-4 as a mesenchymal signal essential for tubule formation provides a strategy for evaluating the role of spinal cord explants as heterologous inducers of kidney tubulogenesis. If the spinal cord mimics a ureteric signal upstream of Wnt-4, this signal would not rescue the mesenchymal requirement for Wnt-4 in tubulogenesis. To test this possibility, isolated metanephric mesenchyme from individual embryos derived from intercrosses between mice heterozygous for a likely null allele of Wnt-4 were cultured on a polycarbonate filter in direct contact with dorsal spinal cord from the same embryo. In the absence of spinal cord, all mesenchyme cultures rapidly degenerated as expected. Surprisingly, when cultured in the presence of spinal cord, mesenchyme from Wnt-4 mutant embryos

developed as well as that of wild-type or heterozygous siblings (Table 1).

Table 1: Induction of tubulogenesis in Wnt-4/Wnt-4 mutant metanephric mesenchyme by dorsal spinal cord

Exp #	# Recombinants	#induced/#total		
		+/+	Wnt-4/+	Wnt-4/Wnt-4
1	8	2/2	5/5	1/1
2	7	1/1	3/3	3/3
3	7	3/3	3/3	1/1
4	5	1/1	3/3	1/1
5	9	3/3	4/4	2/2
6	11	7/7	4/4	-
7	11	3/3	4/4	4/4
Total	58	20/20	26/26	12/12

Isolated metanephric mesenchyme was recombined with dorsal spinal cord from the same embryo on a nucleopore filter. Induction was monitored by bright field microscopy. Embryos of a total of seven litters were analyzed.

Induction of tubulogenesis in wild-type and Wnt-4 mutant metanephric mesenchyme by dorsal spinal cord was analyzed as follows. Isolated metanephric mesenchyme and dorsal spinal cord from the same 11.5 d embryo were recombined on a nucleopore filter. After 48 h and 96 h, cultures were monitored as whole mounts using bright field microscopy; after 144 h, they were analyzed as histological sections. Induction of tubulogenesis in wild-type and Wnt-4/Wnt-4 mutant metanephric mesenchyme were indistinguishable.

After 48 h, induction was visible as bright round zones of condensing mesenchyme. After 96 h, the zones of

condensing mesenchyme had undergone epithelialization to form complex tubules. At 144 h, epithelial tubular structures and glomeruli indicated that full differentiation of induced tubules occurred in all recombinants.

The induction of tubulogenesis in Wnt-4 mutant mesenchyme indicates that spinal cord signaling acts by either mimicking the action of Wnt-4 itself, or a factor downstream of Wnt-4. Further, although Wnt-4 is expressed in the spinal cord, the observation that spinal cord from Wnt-4 mutants is capable of induction indicates that *Wnt-4* expression in the spinal cord is not essential for this process, suggesting the involvement of other Wnts expressed in this tissue.

<u>Wnt polypeptides which are sufficient to trigger tubulogenesis</u>

In order to investigate whether Wnt-4 is sufficient for tubulogenesis, and if this property is shared by other Wnts normally expressed in the spinal cord, NIH3T3 cell lines which stably express various Wnt genes were established. Direct recombinations were performed between Wnt-expressing cells and isolated wild-type metanephric mesenchyme.

Isolated metanephric mesenchyme from 2-3 11.5 d kidneys was placed on top of NIH3T3 cells expressing various Wnt genes. As a control, mesenchymes were placed on NIH3T3 cells expressing LacZ and were placed onto a filter without an underlying cell layer. Induction was scored after 6 d using the morphological appearance of the culture (as documented by brightfield microscopy), and histological analysis of selected samples. For each cell type 2-3 independent experiments were performed.

Induction of tubulogenesis in isolated metanephric mesenchyme by NIH3T3 cells expressing various Wnt genes was evaluated as follows. Brightfield microscopy (24 h,

88 h) and histological analysis (144 h) of direct recombinations between NIH3T3 cells expressing Wnt genes and isolated metanephric mesenchyme. After 24 h, bright zones indicating induction were visible in recombinants between wild-type mesenchyme and Wnt-1, Wnt-3a, Wnt-4, Wnt-7a and Wnt-7b expressing cells. These condensing mesenchymal cells had epithelialized and formed tubular structures after 88 h. After 144 h highly elaborate tubular structures were apparent. In contrast, cells expressing Wnt-5a, Wnt-11, or as a control lacZ, respectively, did not support survival and differentiation of metanephric mesenchyme.

Co-cultures with Wnt-1, Wnt-3a, Wnt-4, Wnt-7a and Wnt-7b expressing cells developed on schedule with those induced by spinal cord and formed complex epithelial tubules with differentiated glomeruli at 144 h (Table 2). In contrast, cells expressing Wnt-5a, Wnt-11 or a lacZ control did not support survival and differentiation of metanephric mesenchyme (Table 2).

<u>Table 2: Induction of tubulogenesis in isolated metanephric mesenchyme by NIH3T3 cells expressing various Wnt genes</u>

Cell line	#induced/#total
Wnt-1	16/16
Wnt-3a	14/14
Wnt-4	14/14
Wnt-5a	0/12
Wnt-7a	12/12
Wnt-7b	11/12
Wnt-11	0/12
LacZ	1/14
mesenchyme	1/12

Wnt mRNA expression was comparable amongst the

various lines. These data indicate that a subset of Wnt genes, which includes Wnt-4 and not Wnt-11, induces tubule formation. As all of these are expressed in the spinal cord at the time of assay, it is likely that these signals account for the robust inducing activity of the spinal cord. However, of these Wnt-4 is the only member which is actually expressed in and which is also required for mesenchymal aggregation.

<u>Wnt-4 triggers the complete program of tubular differentiation</u>

In order to investigate whether Wnt-4 is sufficient to induce fully developed tubules in isolated metanephric mesenchyme, the induction properties of NIH3T3 cells expressing Wnt-4 were analyzed more carefully by assessing the differentiation state of the mesenchyme by histological and molecular criteria.

Histological analysis of tubule induction in isolated metanephric mesenchyme by NIH3T3 cells expressing Wnt-4 was evaluated as follows. NIH3T3 cells expressing Wnt-4 were recombined with isolated metanephric mesenchyme directly and in a transfilter set-up. Cultures were analyzed by sectioning and histological staining after 24 h, 48 h, 96 h and 192 h of culture. Tubule induction in transfilter assays appeared slightly delayed compared to direct recombinations. After 48 h, zones of condensed and aggregated mesenchyme were detected, and after 96 h, epithelial tubules were apparent. After 8 d in culture, fully differentiated tubular structures including glomeruli were detected.

Tubule induction by spinal cord was demonstrated in the art-recognized system in which cells are cultured with polycarbonate filters of a certain pore size (e.g., the method described by Grobstein, 1956, Science 118:52-55). Wnt-4-expressing cells were seeded on one filter; these cells were separated from isolated mesenchyme by

another filter of 1 μm pore size. Induction took place transfilter, though with a delay when compared with direct recombinants.

Transfilter cultures appeared less compact and flatter. Zones of condensed mesenchyme formed after 24 h, and aggregating mesenchyme and simple epithelial bodies appeared after 48 h. Epithelial tubules were seen after 96 h, and glomeruli were detected by 8 days.

To verify that these morphological features reflected an underlying differentiation of the mesenchyme in response to Wnt-4, the temporal and spatial expression of a number of molecular markers was examined. Marker analysis of tubule induction in isolated metanephric mesenchyme by NIH3T3 cells expressing Wnt-4 were analyzed as follows. NIH3T3 cells expressing Wnt-4 were recombined with isolated metanephric mesenchyme in a transfilter set-up and scored for marker expression by *in situ* analysis after 24 h, 48 h, 96 h and 192 h of culture, respectively. Expression of WT-1, Pax-2, Pax-8, Wnt-4 and E-cadherin, respectively, were in accordance with expression data from *in vivo* and *in vitro* studies of tubular differentiation.

WT-1 was broadly expressed after 1 d refining to small intensely labeled foci by 8 days of culture. This expression profile parallels the expression of this gene during metanephric development which is first expressed in condensing mesenchyme, then in simple epithelial bodies before it is restricted to podocytes in the glomeruli. In the recombinants, WT-1 expression was detected in glomeruli after 8 d in agreement with the histological analysis. Like WT-1, Pax-2 is also broadly expressed after 1 d, but becomes restricted to epithelial bodies and is lost after 4 d reflecting initial expression in condensing metanephric mesenchyme, continuing expression in simple epithelial bodies and

subsequent down-regulation as glomeruli start to differentiate. Wnt-4 is expressed in aggregating mesenchyme, in the epithelial bodies which they generate and is subsequently down-regulated as these mature into S-shaped bodies. Pax-8, a paired-box transcription factor, has a similar early expression to Wnt-4 which has been shown to depend on Wnt-4 activity. In cultures, Wnt-4 was transiently expressed between 24 h and 96 h, peaking at 48 h. Pax-8 expression extended longer in S-shaped bodies. E-cadherin, which is expressed in the proximal tubules *in vivo*, was present after 24 h and was maintained, consistent with the differentiation of epithelial tubules along the proximal distal axis.

These data indicate that tubulogenesis in isolated metanephric mesenchyme induced by Wnt-4 follows a similar progression to that observed in the metanephric kidney *in vivo*. At the stage at which the metanephric mesenchyme (T-stage of the ureter) was isolated, initial ureteric signaling had occurred, as evidenced by the condensation of mesenchyme around the tip of the ureteric bud. However, this alone is insufficient to support mesenchymal survival and tubulogenesis. In contrast, Wnt-4 expressing cells were sufficient to support these processes. In order to exclude that Wnt-4 only maintains Wnt-4 expression in the isolated mesenchyme, mesenchyme derived from 10.75 d.p.c. embryos was also analyzed. At this stage, the ureter bud had just emerged and the metanephric mesenchyme can first be identified. Wnt-4 expressing cells triggered the complete differentiation program as judged by brightfield observation (12 out of 12 cases) and by molecular criteria (Pax-8 induction in 8 out of 8 cases after 4 d of culture).

<u>Wnt-4 signaling requires cell contact</u>

Tubule induction in isolated metanephric mesenchyme was analyzed with respect to filter pore size.

Experiments using the spinal cord as a heterologous inducer suggest a requirement for cell-cell contact as pore sizes below 0.1 µm, which prevent the extension of cytoplasmic processes, block induction.

Pore size dependence of tubule induction by Wnt-4 expressing cells was tested as follows. NIH3T3 cells expressing Wnt-4 were recombined with isolated metanephric mesenchyme in a transfilter set-up with various pore sizes of the nucleopore filter. Induction was scored after 4 d by Pax-8 expression in whole mount *in situ* analysis. Pore sizes of 0.1 µm and bigger supported full induction of metanephric mesenchyme, whereas 0.05 µm pore size reduced or abolished induction (Table 3).

Table 3: Induction of tubulogenesis in isolated metanephric mesenchyme by NIH3T3 cells expressing Wnt-4 in transfilter assays with increasing pore size

Pore size	# induced/# total
0.05 µm	3*/13
0.1 µm	14/16
0.4 µm	14/14
0.8 µm	6/6
1 µm	3/3

* In each of the specimen scored as induced, only 1-4 spots of Pax-8 expression were seen in contrast to 15-30 with all the other pore sizes.

Supernatants from Wnt-4 expressing cells alone did not induce tubulogenesis, suggesting that cell contact is required. Wnt-4 may act as an insoluble cell bound factor or it may associate with the extracellular matrix (ECM). It is unlikely that Wnt-4 mediated induction occurs through a secondary, soluble factor.

Wnt-4 signaling requires sulphated glycosaminoglycans

Experiments were carried out to determine whether Wnt signaling for tubule induction depends on sulfated glycosaminoglycans (GAG)s which might act as cofactors for binding the Wnt protein on the responsive cell. Accordingly, studies were undertaken to see evaluate whether the presence of 30 mM $NaClO_3$ (a competitive inhibitor of sulfation of GAGs) affects tubule induction. NIH3T3 cells expressing Wnt-4 were recombined with isolated metanephric mesenchyme in a transfilter set-up with addition of 30 mM $NaClO_3$ in the medium. $NaClO_3$ was added to cultures at the start of transfilter culture, or 24 and 48 h later. As a control, chlorate was omitted completely. Induction was scored after 4 d by Pax-8 expression using whole mount *in situ* hybridization analysis. Addition of 30 mM $NaClO_3$ after 24 h or 48 h of culture did not affect tubule induction compared to untreated controls, whereas administration of 30 mM $NaClO_3$ at the beginning of the culture abrogated tubule induction completely (Table 4).

Table 4: Induction of tubulogenesis in isolated metanephric mesenchyme by NIH3T3 cells expressing Wnt-4 in presence of 30 mM $NaClO_3$

30 mM $NaClO_3$ added after h in culture	#induced/#total
0 h	0/19
24 h	12/19
48 h	14/17
-	12/15

When chlorate was added at 0 h, mesenchyme degenerated and Pax-8 expression was consequently negative. However, addition of chlorate after 24 h did

not influence Pax-8 expression. Hence, GAGs are not involved in tubule maturation and differentiation. Tubule induction does, however, depend on sulfated GAGs in the first 24 h, the period essential for complete induction by the spinal cord.

The chlorate inhibition experiments define a critical period of 24 h for induction. Further differentiation, i.e. aggregation and epithelialization of mesenchymal cells is only initiated when a certain number of cells (a small community) has received the Wnt-4 signal. At this time, mesenchymal development is independent of ureteric signaling.

Chlorate acts as a competitive inhibitor of sulphotransferases and inhibits the sulphation of glycosaminoglycans. The inhibition studies point to a critical role of these ECM compounds in tubulogenesis. Numerous studies have shown that branching morphogenesis of the ureter as well as branching of other epithelia requires an intact ECM. Since presence of chlorate after 24 h does not influence tubulogenesis, GAGs do not seem to be involved in tubule maturation and differentiation. Tubule induction does, however, depend on sulfated GAGs in the first 24 h, the period essential for complete induction by the spinal cord. GAGs may act as co-receptors, facilitating presentation or increasing the local concentration of the ligand.

Wnt-4 signaling as a trigger for tubulogenesis

In order to test whether Wnt-4 expressing cells can rescue a Wnt-4 mutant mesenchyme, direct recombination experiments were carried out in culture. Induction of tubulogenesis in wild-type and Wnt-4 mutant metanephric mesenchyme by NIH3T3 cells stably expressing Wnt-4 was evaluated as follows. Isolated metanephric mesenchyme was placed on top of NIH3T3 cells expressing Wnt-4 which were supported by a nucleopore filter. After

48 h and 96 h, cultures were monitored as whole mounts using bright field microscopy; after 144 h, the cultures were monitored as histological sections. Induction of tubulogenesis in wild-type and Wnt-4/Wnt-4 mutant metanephric mesenchyme by Wnt-4 expressing cells were indistinguishable. Wnt-4-expressing cells were equally efficient at inducing tubule formation in wild type or Wnt-4 mutant metanephric mesenchyme (Table 5).

Brightfield microscopy and histological analysis of specimen After 6 d in culture revealed the full spectrum of tubular differentiation including glomerulus formation.

As with spinal cord mediated induction, Wnt-4 expression in the mesenchyme itself is not required for tubule formation, but supplying Wnt-4 in adjacent cells is sufficient to trigger the inductive process. These results suggest that whereas Wnt-4 plays an essential role in initial tubulogenesis, it may not be required for later morphogenesis of the tubule. As shown in Table 5, Wnt-1 expressing cells were also sufficient to trigger tubulogenesis in mesenchyme mutant for Wnt-4.

Table 5: Induction of tubulogenesis in Wnt-4/Wnt-4 mutant metanephric mesenchyme by NIH3T3 cells expressing Wnt-4 or Wnt-1

#Exp	# Recombinants	#induced/#total		
		+/+	Wnt-4/+	Wnt-4/Wnt-4
with NIH3T3 cells expressing Wnt-4:				
4	42	7/7	18/18	17/17
with NIH3T3 cells expressing Wnt-1:				
2	20	5/5	11/12	3/3

Mammalian kidney development

Metanephric development is a highly coordinated process characterized by a continuous interaction of the epithelial ureter and the surrounding metanephric mesenchyme. Classical organ culture experiments have pointed to the fact that these two compartments achieve coordinated development by use of reciprocal signaling systems. First, the metanephric blastema induces a bud from the adjacent nephric duct which invades and branches into the mesenchyme. This process appears to be mediated by GDNF which is secreted by the metanephric mesenchyme and sensed by the c-ret/GDNFRa receptor complex on the ureter. Next, the metanephric mesenchyme undergoes tubulogenesis upon a permissive stimulus from the ureter.

Signals required for induction of tubulogenesis

In addition to Wnt-4, other Wnts may replace Wnt-4 activity in the mesenchyme. Using cell lines expressing various Wnt genes, Wnt-1, Wnt-3a, Wnt-7a, Wnt-7b, were

shown to evoke tubulogenesis in isolated metanephric mesenchyme. The results described herein suggest a different interpretation of the use of kidney cultures to elucidate the nature of the ureteric signal involved in inducing the mesenchyme. Experiments which have used heterologous sources of tubule inducers, e.g., the spinal cord, may not have been investigating the nature of ureteric signaling, but rather the mesenchymal action of signals such as Wnt-4. At present, the exact nature of ureteric signaling remains obscure. A primary signal might be required for a sufficient length of time to allow auto-induction of the mesenchyme by Wnt-4. Alternatively, a secondary signal from the ureter tip might be necessary to induce Wnt-4 expression in aggregating mesenchyme. In contrast to earlier studies, the data presented herein indicate that Wnt-11 does not play a role as a ureteric signal for mesenchymal aggregation. In the present studies, tubulogenesis was not detected with cells expressing Wnt-11.

<u>Wnt-4 is a mesenchymal signal for tubulogenesis</u>

Analysis of Wnt-4 mutants has demonstrated a critical role for Wnt-4 in kidney development. Homozygous pups die 24 h after birth due to small agenic kidneys consisting of undifferentiated mesenchyme intermingled with collecting duct tissue. Histological and marker analysis revealed that primary condensation of mesenchymal cells around the ureter tips as well as ureteric branching occurs normally. However, mutant kidneys quickly become growth retarded and the mesenchyme remains undifferentiated lacking pretubular cell aggregates and epithelial tubules. Since kidney size as well as cell death initially remain unaffected, proliferation is unlikely to be controlled by Wnt-4. Rather, the lack of Wnt-4 expression itself and of epithelial structures in the mutant mesenchyme indicates

that Wnt-4 may autoinduce the epithelialization of condensed mesenchyme. Mesenchymally-derived Wnt-4 is not only required but also sufficient for induction of tubulogenesis in the mammalian kidney. Judging by histological and molecular markers, Wnt-4 can elicit the complete program of tubular differentiation in isolated metanephric mesenchyme. The activity of Wnt-4 contrasts with other factors thought to regulate mesenchymal development. For example, basic fibroblast growth factor (FGF) and epidermal growth factor (EGF) can both support mesenchymal survival but are not sufficient for tubulogenesis. Like Wnt-4, BMP-7 has been suggested to induce tubules, but loss-of-function studies indicate it is not essential for tubule formation in vivo as some glomeruli form in BMP7 mutants. In contrast, loss of Wnt-4 led to a complete absence of glomeruli.

Wnt-4 activity shows all the characteristics which have previously been ascribed to induction by dorsal spinal cord tissue. Signaling is cell-contact dependent. Below a certain pore size in the transfilter assay the formation of cellular processes which penetrate the filter pores is inhibited and isolated mesenchyme degenerates. Cell contact is required for induction of tubulogenesis, and Wnt proteins may interact with extracellular matrix (ECM) components.

Wnt-4 expression in the metanephric mesenchyme is initiated in the aggregating mesenchyme and maintained in the comma shaped bodies before it is downregulated in S-shaped bodies. Therefore, Wnt-4 likely has a later function in tubulogenesis.

Table 15: Human Wnt-4-encoding nucleic acid

1	TGCAAGTGTC	ACGGGGTGTC	AGGCTCCTGT	GAGGTAAAGA	CGTGCTGGCG
51	AGCCGTGCCG	CCCTTCCGCC	AGGTGGGTCA	CGCACTGAAG	GAGAAGTTTG
101	ATGGTGCCAC	TGAGGTGGAG	CCACGCCGCG	TGGGCTCCTC	CAGGGCACTG
151	GTGCCACGCA	ACGCACAGTT	CAAGCCGCAC	ACAGATGAGG	ACCTGGTGTA
201	CTTGGAGCCT	AGCCCCGACT	TCTGTGAGCA	GGACATGCGC	AGCGGCGTGC
251	TGGGCACGAG	GGGCCGCACA	TGCAACAAGA	CGTCCAAGGC	CATCGACGGC
301	TGTGAGCTGC	TGTGCTGTGG	CCGCGGCTTC	CACACGGCGC	AGGTGGAGCT
351	GGCTGAACGC	TGCAGCTGCA	AATTCCACTG	GTGCTTGTTC	TTGAGTCGAC

SEQ ID NO: 10

Table 16: Human Wnt-7a-encoding nucleic acid

1	TGTAAGTGTC	ACGGCGTGTC	AGGCTCGTGC	ACCACCAAGA	CGTGCTGGAC
51	CACACTGCCA	CAGTTTCGGG	AGCTGGGCTA	CGTGCTCAAG	GACAAGTACA
101	ACGAGGCCGT	TCACGTGGAG	CCTGTGCGTG	CCAGCCGCAA	CAAGCGGCCC
151	ACCTTCCTGA	AGATCAAGAA	GCCACTGTCG	TACCGCAAGC	CCATGGACAC
201	GGACCTGGTG	TACATCGAGA	AGTCGCCCAA	CTACTGCGAG	GGGGACCCGG
251	TGACCGGCAG	TGTGGGCACC	CAGGGCCGCG	CCTGCAACAA	GACGGCTCCC
301	CAGGCCAGCG	GCTGTGACCT	CATGTGCTGT	GGGCGTGGCT	ACAACACCCA
351	CCAGTACGCC	CGCGTGTGGC	AGTGCAATTG	TAAGTTCCAT	TGGTGC

SEQ ID NO: 11

Table 17: Human Wnt-7b-encoding nucleic acid

```
  1  GTAAAATGTC ACGGCGTGTC TGGCTCCTGC ACCACCAAAA CCTGCTGGAC
 51  CACGCTGCCC AAGTTCCGAG AGGTGGGCCA CCTGCTGAAG GAGAAGTACA
101  ACGCGGCCGT GCAGGTGGAG GTGGTGCGGG CCAGCCGTCT GCGGCAGCCC
151  ACCTTCCTGC GCATCAAACA GCTGCGCAGC TATCAGAAGC CCATGGAGAC
201  AGACCTGGTG TACATTGAGA AGTCGCCCAA CTACTGCGAG GAGGACGCGG
251  CCACGGGCAG CGTGGGCACG CAGGGCCGTC TCTGCAACCG CACGTCGCCC
301  GGCGCGGACG GCTGTGACAC CATGTGCTGC GGCCGAGGCT ACAACACCCA
351  CCAGTACACC AAGGTGTGGC AGTGCAACTG CAAATTCCAC TGGTGCTGCT
401  CTAG
```

SEQ ID NO: 12

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

What is claimed is: